Biologic Effects of Oil Fly Ash

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Epidemiologic studies have demonstrated increased human morbidity and mortality with elevations in the concentration of ambient air particulate matter (PM). Fugitive fly ash from the combustion of oil and residual fuel oil significantly contributes to the ambient air particle burden. Residual oil fly ash (ROFA) is remarkable in the capacity to provoke injury in experimental systems. The unique composition of this emission source particle makes it particularly useful as a surrogate for ambient air PM in studies of biologic effects testing the hypothesis that metals mediate the biologic effects of air pollution particles. A majority of the *in vitro* and animal model investigations support the postulate that transition metals present in ROFA (especially vanadium) participate in Fenton-like chemical reactions to produce reactive oxygen species. This is associated with tyrosine phosphorylation, nuclear factor kappa B and other transcription factor activation, induction of inflammatory mediator expression, and inflammatory lung injury. It is also evident that vanadium accounts for a significant portion of the biologic activity of ROFA. The extrapolation of this body of investigation on ROFA to the field of ambient air PM is difficult, as particles in numerous environments have such small amounts of vanadium. *Key words*: air pollution, lung diseases, oxidative stress, vanadium. *Environ Health Perspect* 110(suppl 1):89–94 (2002).
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Epidemiologic studies have demonstrated increased human morbidity and mortality (*1*) with elevations in the concentration of ambient air particulate matter (PM). Fine particles, with mass median aerodynamic diameters (MMAD) less than 2.5 μm, are more closely associated with adverse health effects of PM than coarser particles (*2*). Fine particles often represent anthropogenic sources, as they frequently result from an incomplete oxidation of carbonaceous materials. The inorganic residue that remains after burning such a substance is termed "fly ash." Fly ash from fossil and waste fuel combustion contributes more than 2.5×10^5 tons annually to the ambient air PM burden in the United States (*3*). Although the ash content of oil used for electric power generation is two to three orders of magnitude less than that of coal, many oil-fired power plants employ few or no particle emission abatement technologies (*4*). Consequently, fugitive fly ash from the combustion of oil and residual fuel oil contributed 76,000 and 49,000 tons, respectively, to the national ambient particle burden in 1992 (*5*).

Oil fly ash is frequently less than 2.5 μm in MMAD. This combustion product is principally inorganic. However, analyses of fly ashes are performed after complete oxidation of the sample to stable metal oxides. This can lead to the incorrect conclusion that fly ashes are simple, insoluble, and unreactive pollutant products. Comparable to ambient air pollution particles, oil fly ash is chemically complex and includes sulfates, silicates, carbon- and nitrogen-containing compounds, contaminants of the fuel, and additives. Metals, including iron, vanadium, and nickel, are present in high concentrations as water-soluble salts in fly ash (*6*).

Tropospheric concentrations of vanadium are often employed as a marker of the contribution of oil fly ash to the total PM level in an air shed (*6*). In a rural setting, vanadium in ambient air can vary between 25 and 75 ng/m^3, whereas in an urban environment, this value is 60–300 ng/m^3 and can increase 6-fold in the winter (*7*). Although this metal is an essential trace element for humans and certain animals, it occurs sparsely in nature (*8*). Certain plants can have higher levels of vanadium (e.g., sugar beets, vines, beech and oak trees), but the greatest concentrations are found in lower marine animals (e.g., shellfish) (*8*). Because oil is derived from fossilized marine organisms, vanadium is found in this fuel at a high average concentration and, subsequently, in its fly ash (Figure 1A,B). Higher contents of the metal occur in the heavy oils left (i.e., the residual) after the more volatile fractions such as petrol, paraffin, and diesel oil have been distilled, hence the term "residual oil" fly ash (ROFA) (*8*). In addition to metals, sulfates are in abundance (Figure 1A,C).

ROFA is remarkable in its capacity to induce lung injury in experimental animal models (*9*). Because ROFA is rich in metals, with little organic component, it has been particularly useful as a surrogate for ambient air PM in studies of biologic effect, testing the hypothesis that metals mediate the biologic effects of air pollution particles.

The mechanism of lung injury after exposure to ambient air PM is not known. Injury has been postulated to be mediated either by metal-catalyzed oxidant generation or by metal ion dysregulation of phosphotyrosine metabolism, or possibly elements of both mechanisms (Figure 2) (*10*). These events are then proposed to result in phosphorylation-dependent cell signaling, an activation of specific transcription factors such as nuclear factor kappa B (NFκB) and AP-1, an increased expression of proinflammatory proteins whose genes have binding sites for these transcription factors in their promoter regions, and finally leading to an inflammatory injury to the lung. We review the biologic effects of oil fly ash on both cells and tissues, specify the association of this effect with vanadium, and opine on the relevance of this investigation to human morbidity and mortality following exposure to air pollution particles.

Effects of Residual Oil Fly Ash on Cells

Epithelial cells of the respiratory tract not only function to provide a structural barrier to inhaled agents but are now recognized as critical participants in the function of the lung, partly through their production of inflammatory mediators. Epithelial cells take up ROFA into pits/vesicles that appear to be clathrin-coated (Figure 3A,B). The particle then generates oxygen-based free radicals to present an oxidative stress to the cell (*11,12*) comparable that in to acellular systems (*9*). This cellular production of oxidants can be inhibited by either the metal chelator deferoxamine or the antioxidants *N*-acetylcysteine and dimethylthiourea (DMTU). Exposure to the ash results in increased epithelial permeability, cell detachment, and a lytic injury

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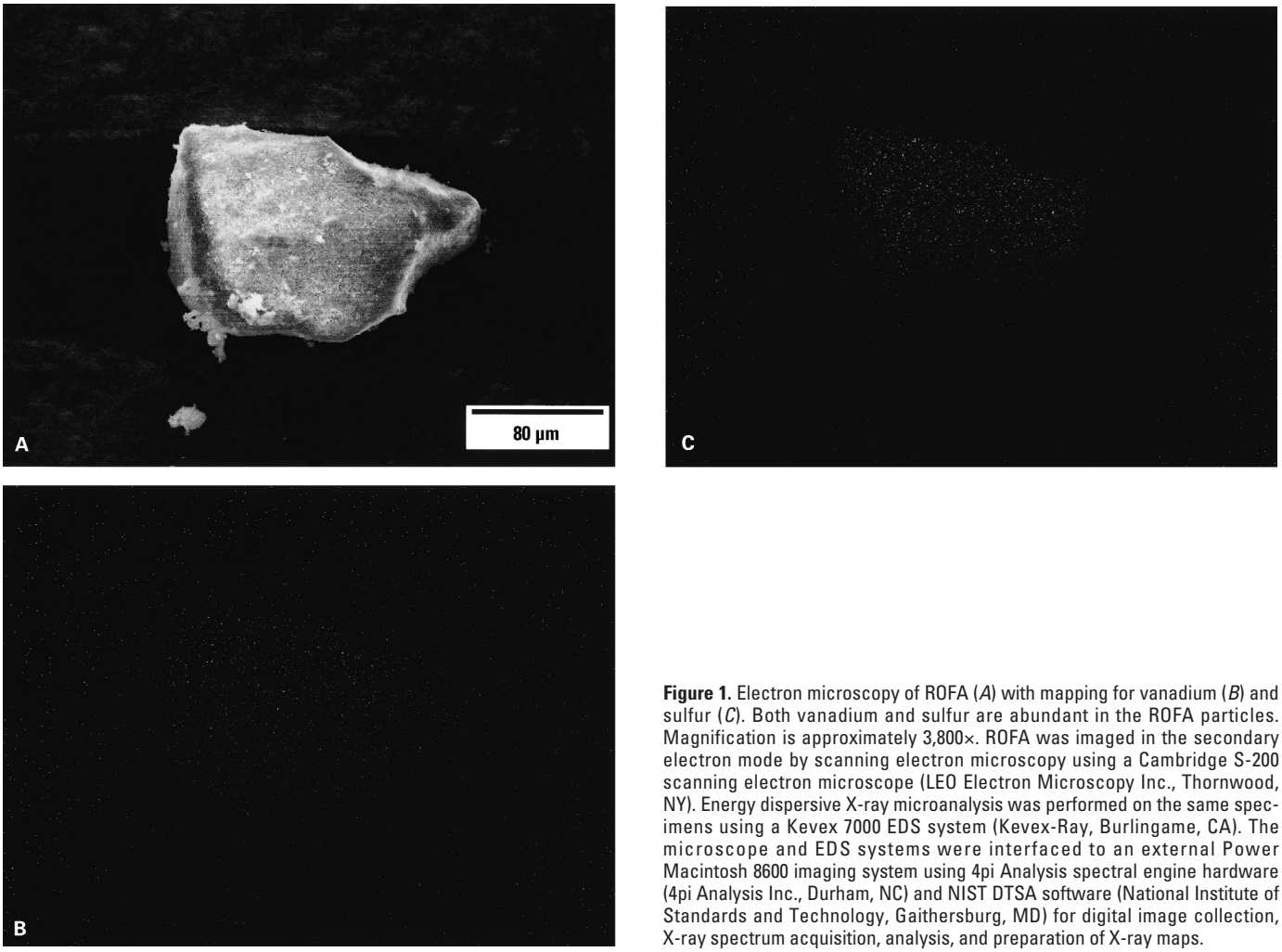

Figure 1. Electron microscopy of ROFA (*A*) with mapping for vanadium (*B*) and sulfur (*C*). Both vanadium and sulfur are abundant in the ROFA particles. Magnification is approximately 3,800×. ROFA was imaged in the secondary electron mode by scanning electron microscopy using a Cambridge S-200 scanning electron microscope (LEO Electron Microscopy Inc., Thornwood, NY). Energy dispersive X-ray microanalysis was performed on the same specimens using a Kevex 7000 EDS system (Kevex-Ray, Burlingame, CA). The microscope and EDS systems were interfaced to an external Power Macintosh 8600 imaging system using 4pi Analysis spectral engine hardware (4pi Analysis Inc., Durham, NC) and NIST DTSA software (National Institute of Standards and Technology, Gaithersburg, MD) for digital image collection, X-ray spectrum acquisition, analysis, and preparation of X-ray maps.

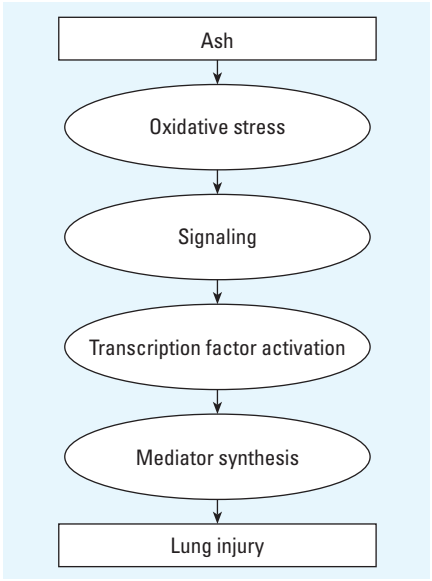

Figure 2. Schematic of the proposed mechanism for the biologic effect of ROFA.

with release of lactate dehydrogenase (*13*). The dependence of these cytotoxic effects of ROFA on the generation of an oxidative stress is supported by the protective effect of the antioxidant DMTU on cell permeability after exposure to this emission source PM (*13,14*).

As mentioned earlier, incubation of respiratory epithelial cells with ROFA is associated with the initiation of phosphorylation-dependent signaling reactions that may be modulated by specific redox changes (*15*). Interestingly, redox-active vanadium compounds can reproduce these events, whereas catalytically active iron and nickel compounds have no effect (*15*). One transcription factor known to be associated with oxidant responses is NFκB. NFκB is normally sequestered in the cytoplasm as an inactive multiunit complex bound by the inhibitory protein IκB. In the nucleus, NFκB binds to promoter and enhancer regions of a multitude of genes involved in the inflammatory response, including cytokines, chemokines, and growth factors. It is postulated that these genes then function to initiate, amplify, and coordinate the inflammatory response. ROFA induces phosphorylation and degradation of IκB, with a resulting translocation of the active dimer into the nucleus in respiratory epithelial cells (*16*). ROFA-induced activation of NFκB is blocked by metal chelators and free radical scavengers, suggesting that this activation is dependent on the generation of oxidants (*16*).

Respiratory epithelial cells exposed to either ROFA or vanadium, but not iron or nickel, showed increased messenger RNA (mRNA) and protein expression of numerous cytokines, including interleukin (IL)-6, IL-8, and tumor necrosis factor (*17*). In addition, prostaglandin H synthase 2 expression is induced, and there is concomitant enhanced secretion of prostaglandins E_2 and $F_{2\alpha}$ from normal human airway epithelial cells exposed to ROFA (*18*). As with NFκB activation, deferoxamine and an antioxidant diminish the release of inflammatory mediators induced by ROFA in these cells (*17*).

Similar to epithelial cells, ROFA exposure can result in an intracellular oxidant stress within alveolar macrophages, which

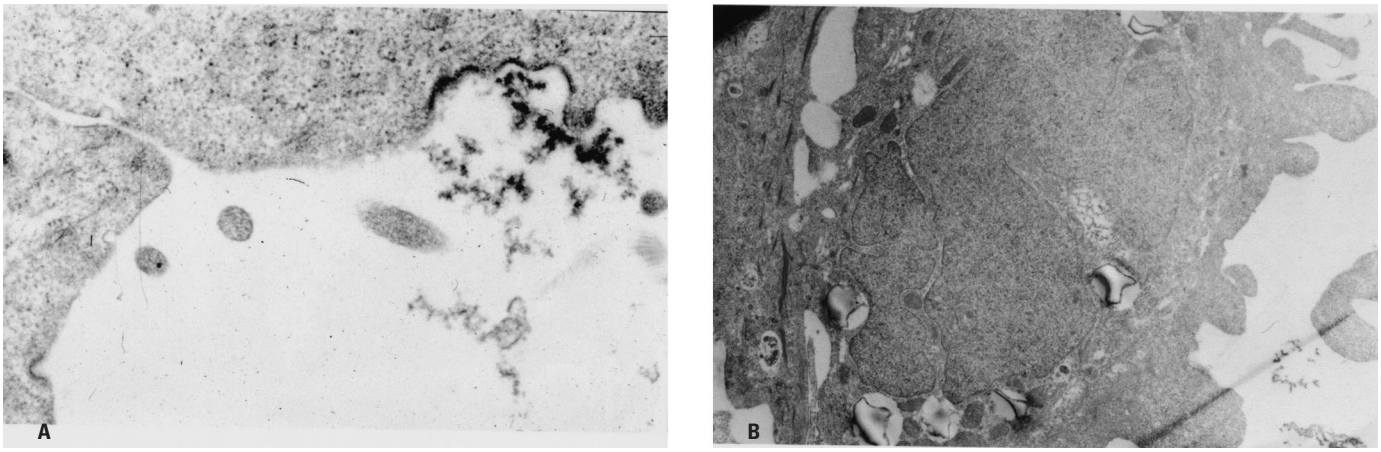

Figure 3. Incubation of BEAS-2B cells (90–100% confluence) in plastic, 12-well plates (KGM; Clonetics, San Diego, CA) with 200 µg ROFA/mL for 24 hr results in uptake of particulate components of the ash. Coated pits are evident at the membrane (*A*) and putative clathrin-coated vesicles are shown with components enclosed (*B*). Magnification is approximately 1,500×.

may contribute to cellular activation and production of proinflammatory mediators (*19–21*). The primary source of these oxidants is likely the NADPH oxidase activity in the alveolar macrophage itself. However, transition metals may also present direct oxidative stress to this cell (*22*). Relative to the epithelial cells, cytotoxicity appears to occur at lower particle concentrations, and the magnitude of cytokine release is reduced (*23*). Comparable to respiratory epithelial cells, the oxidant burst and mediator release after incubation of macrophages with ROFA appears to be driven by vanadium and can be inhibited by deferoxamine and/or antioxidants, suggesting both intrinsic and extrinsic sources for radical generation by alveolar macrophages (*19,21*).

Physiologic, Biochemical, Cellular, and Molecular Effects of Residual Oil Fly Ash in Animals

In vivo oxidative stress in the lungs of animals instilled with ROFA has been verified by electron spin resonance (ESR) (*24*). This ESR signal can be reproduced by treatment with vanadium, but not iron or nickel compounds (*24*). The activation of both phosphorylation-dependent kinases (i.e., mitogen-activated protein kinases) in the rat lung following instillation with ROFA has been reported (*25*). Figure 4 shows the effect of ROFA on the translocation of the NFκB subunit P65 and phosphorylation of the transcription factors cAMP-responsive element binding, activating transcription factor 2, and c-Jun in the rat lung. Furthermore, mRNA and protein expression of mediators of inflammation and fibrosis are also elevated in these tissues following ROFA instillation (*26*). Finally, exposure to the emission source particles results in a dose-dependent influx of inflammatory cells (*9,27*). This is almost always neutrophilic, but occasional eosinophilic infiltration into the lower respiratory tract has been noted (*27*). The peak of this influx occurs 18–24 hr after exposure.

Injury to the lung in the animal is evident within 24 hr of exposure, with detachment of ciliated and mucus cells from the epithelial lining of the terminal bronchioles accompanied by hemorrhage. Inhalation or instillation of an equivalent concentration of ROFA evokes pulmonary inflammatory responses that are qualitatively and quantitatively similar (*28*). Whereas incursion of inflammatory cells appears to best correlate with vanadium exposure, injury assessed as protein concentrations in the lavage fluid correlates best with the nickel content in ROFA (*29*). The cellular influx persists 96 hr later, and resolution occurs slowly (*9*). Instillations of larger amounts of oil fly ash (500 µg or greater) can induce a rapid onset of noncardiogenic pulmonary edema (*30*).

Exposures to an aqueous extract of ROFA produce effects very similar to those induced by unfractionated ROFA (*31*). In marked contrast, the water-insoluble component has minimal effect on the lungs of the rats (*31*). In animal models of compromised cardiopulmonary function (e.g., spontaneously hypertensive rats), lung injury after ROFA can be considerably more extensive (*3,32,33*). Pulmonary inflammatory injury induced by ROFA is reproducible by instillation of a mixture of soluble forms of vanadium, nickel, and iron in the proportions found in a saline leachate of ROFA (*31*). Pretreatment with DMTU significantly decreased the number of neutrophils present in bronchoalveolar lavage fluid, further supporting metal-catalyzed oxidative stress as a factor determining inflammatory injury after ROFA instillation in animals (*13*). Finally, animals exposed to vanadium-containing compounds demonstrate neutrophilic inflammatory injury of the bronchi and the distal lung accompanied by a significant airflow limitation, confirming that it is a significant determinant of injury presented by ROFA in the respiratory system (*34*). These results suggest that transition metals play a key role in mediating the injury seen after ROFA instillation.

Inflammatory lung injury after ROFA is accompanied by airway hyperreactivity (*35*) and an increase in susceptibility to infections (*9*) in normal animals. The metal composition of the ash appears critical to the development of airway hyperreactivity, as assessed by acetylcholine challenge (*9*). In addition, there are effects of ROFA on sensitization to allergens in animal models of pulmonary allergy, with significant elevations in eosinophils, IL-10, antigen specific immunoglobulin E, and associated immediate bronchoconstriction responses to antigen challenge (*36–39*). This effect can be reproduced by the metal leachate of ROFA as well as individual metallic constituents of ROFA (*35*) and can be abrogated by DMTU pretreatment (*40*). These results suggest that the oxidative stress presented by metals present in ROFA is responsible for the airway hyperreactivity and sensitization to allergens (*41*).

There are also effects of ROFA exposure on heart function, with a bradycardic response in healthy animals that persists up to 48 hr after instillation, implicating both conductive and hypoxemic arrhythmogenic mechanisms leading to cardiac-related deaths (*30*). A related extrapulmonary impact of ROFA is an elevation in plasma concentrations of fibrinogen in exposed rats (*42*).

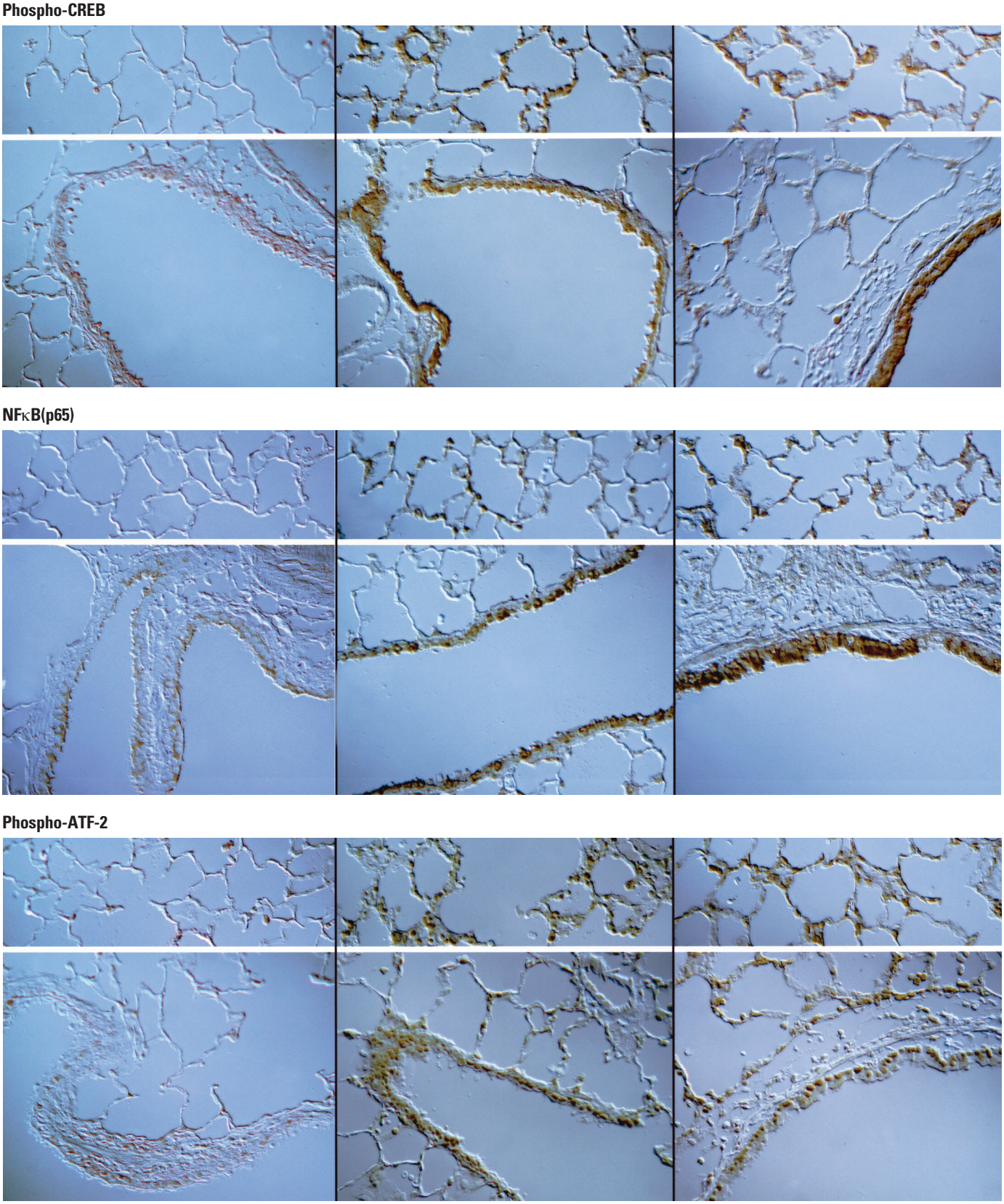

Figure 4. Transcription factor activation in lung tissue following instillation of ROFA. Male Sprague-Dawley rats were intratracheally instilled with 0.5 mL saline alone (left panels) or saline containing 0.5 mg ROFA for 4 hr (middle panel) or 24 hr (right panel). Lungs were formalin-fixed, paraffin-embedded, sectioned, and immunostained using anti-P65, anti–phospho-CREB, anti–phospho-ATF-2 or anti–phospho-c-Jun antibodies. Four hr after the treatment, pronounced immunostaining for all transcription factors is seen along the airway epithelium and parenchymal (insets) tissue is evident. Consistent with the mechanism of activation, P65 and P-ATF-2 staining appear to be nuclear and perinuclear. (*Continued on next page*)

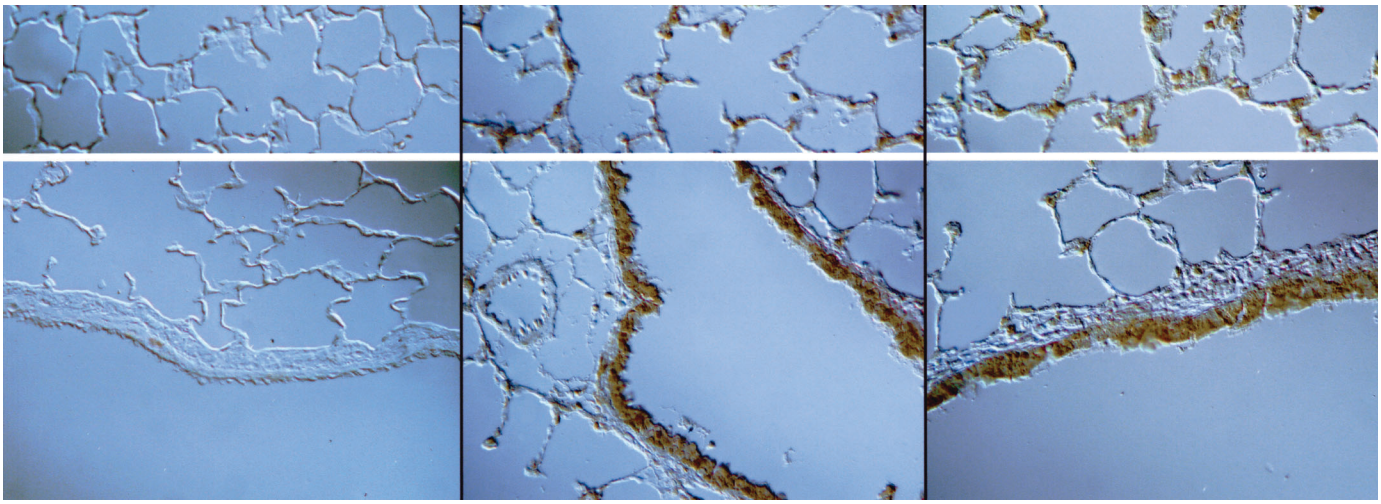

Figure 4. Continued.

Human Injury after Exposure to Residual Oil Fly Ash

Relative to animal models, considerably less is known about the effects of injury in humans after exposure to ROFA. Lung injury after human exposure to oil fly ash has occurred predominantly after occupational exposures of workers engaged in the maintenance of oil-fired boilers in power generating stations (*43–49*). The clinical presentation of these workers has been termed "boilermakers' bronchitis" or "vanadium bronchitis." Individuals exposed to high concentrations of ash provide a history of eye irritation, sore throat, hoarseness, cough, dyspnea, wheezing, and, infrequently, symptoms consistent with pneumonitis. Physical examination reveals rhinitis, conjunctivitis, and wheezing. Within 24 hr of exposure to ROFA, dose-dependent decreases in indices of pulmonary function have been observed, including diminished forced vital capacity, forced expiratory volume in 1 sec, and forced expiratory flows (*48,49*). Bronchoscopic examination shows a bronchitis with erythema and discharge in ROFA-exposed individuals. Symptoms and signs subside, and pulmonary function decrements can resolve, within a few days or weeks of cessation of the exposure (*47*).

As a result of the comparable clinical presentations, physiology, and pathology of injury after ROFA and vanadium exposures, it has been suggested that this metal is a major component responsible for toxicity of this air pollution particle in humans. Vanadium pentoxide (V_2O_5) is widely used as a catalyst for a variety of reactions and in the production of high-strength steel alloys (*50*). The first report of a human exposure to vanadium was in 1911 (*50*). "Vanadiumism" was defined as a chronic intoxication with the principal evidence of toxicity observed in the lungs, kidneys, and gastrointestinal tract (*50*). Exposures occur during the mining, separation, and use of V_2O_5 in the steel and chemical industries. Exposure occurs mostly via inhalation, and vanadium is excreted in the urine, with a smaller amount in the feces. Vanadium dust causes symptoms of respiratory tract irritation with conjunctivitis, sneezing, rhinorrhea, sore throat, and chest tightness (*50–52*). The cough is prominent and characteristically dry and paroxysmal (*50*). Examination of vanadium-exposed individuals reveals a greenish discoloration of the tongue, wheezing, rhonchi, and rales (*51,52*). An increase in the inflammatory cells in nasal smears and biopsies from the nasal mucosa accompanies symptoms of respiratory tract irritation. There can be concurrent changes in pulmonary function indices associated with vanadium exposure (*51,52*). Vanadium workers are more susceptible to tuberculosis and can rapidly succumb to this disease (*50*). At high exposure levels, the lungs become highly congested and show a marked destruction of the alveolar epithelium (*50*). At high vanadium exposures, hemorrhages are frequent and severe, even causing death (*51,52*). Workers who died from vanadium exposure showed congested lungs with destruction of the alveolar epithelium (*51,52*).

Relevance to Injury after Ambient Particulate Matter

At the cellular level and in animal models, a majority of the investigation supports the postulate that transition metals found in ROFA (especially vanadium) participate in Fenton-like chemical reactions to produce reactive species. This is associated with tyrosine phosphorylation, intracellular signaling leading to NFκB translocation and activation of other transcription factors, induction of inflammatory mediator expression, and inflammatory lung injury. Cardiac and systemic effects result from a dissemination of components of ROFA to extrapulmonary tissues, reflexes, or hypoxemia. It is also evident that vanadium in ROFA accounts for the greatest portion of this effect. Vanadium as a contributor to the toxicity of air pollution particles may be specific to ROFA. In support of a potential role of vanadium in the biologic effect of ambient air PM, epidemiologic studies have shown a correlation between vanadium levels in the air and the incidence of mortality from bronchitis and pneumonia in British cities (*53*). However, an extrapolation of the body of investigation on ROFA to the health effects of ambient air PM is complicated by the fact that ambient air PM collected from numerous environments has very small amounts of vanadium. Despite this limitation, data continue to accumulate, suggesting that ambient air and other emission source particles follow a comparable mechanism of action as ROFA, involving phosphorylation reactions (*54*), transcription factor activation (*54*), mediator release (*55*), and inflammatory injury (*56*).

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